REMARKS

Compliance with the Sequence Listing Requirements

The specification has been objected to for failing to comply with the Sequence Listing Requirements under 37 C.F.R. §§1.821-1.825. Enclosed herewith in full compliance with 37 C.F.R. §§1.821-1.825 is a Sequence Listing to be inserted into the specification as indicated above. The Sequence Listing in no way introduces new matter into the specification.

Also submitted herewith in full compliance with 37 C.F.R. §§1.821-1.825 is a disk copy of the Sequence Listing. The disk copy of the Sequence Listing, file "1209-121.app", is identical to the paper copy, except that it lacks formatting. Withdrawal of the objection is, therefore, respectfully requested.

Rejections under 35 U.S.C. §112, second paragraph

The claims have been rejected under 35 U.S.C. §112, second paragraph as being indefinite, for the following reasons.

1) Claims 1-8 have been rejected for recitation of "characterized in that."

2) Claims 6 and 7 have been rejected as being unclear in the respective recitation of "crosslinkable oligonucleotides" and "a ligase is added," with the assertion that it is not clear whether the addition of the ligase is a positive effect.

3) Claim 8 has been rejected for recitation of "an oligonucleotide complementary to the crosslinkable oligonucleotides of claims 6 or 7...."

4) Claim 6 has been further rejected for recitation of "amplifying said crosslinked oligonucleotides" with the assertion that it is not clear when or how the oligonucleotides became crosslinked.

5) Claims 1 and 4 have been rejected for being drawn to an immunological test kit, but failing to recite an antibody.

6) Claim 3 has been rejected as being unclear and improperly reciting a Markush group.

The claims have been amended as indicated above to address the rejections raised by the Examiner and clarify the scope of the claims. As the above amendments address the rejections of the claims as being indefinite, withdrawal of the rejections is respectfully requested.

Rejections under 35 U.S.C. §102

Claims 1-5 have been rejected as being anticipated by or being obvious over Urdea et al. (U.S. Patent No. 5,656,731). Urdea et al. is asserted to teach nucleic acid-amplified immunoassay reagents in the kits of the present invention. Ureda et al. is further asserted to differ from the present invention only in failing to claim the reagents as a kit. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

The present invention is drawn to a test kit comprising

a) a first immobilized reagent having affinity to a specific macromolecule, and

b) a second and a third affinity reagent specific for different determinants of said macromolecule, and modified with crosslinkable oligonucleotides, wherein a signal is generated when said second and third affinity reagents are bound to the same macromolecule.

Urdea et al. disclose reagents wherein an analyte specific domain has been combined with a nucleic acid sequence. This combination allows signal amplification through the transcription of the nucleic acid sequence. The present invention relies on the concept of a signal being generated when two detection probes are bound

sufficiently close to each other. There is no disclosure or suggestion in Urdea et al. of creating a signal by the ligation of two sufficiently closely bound detection probes. The ligation reaction disclosed in Urdea et al. pertains to the manufacture of the probe, not to the assay conditions/reactions. As such the present invention is distinct from and not obvious over Urdea et al. Withdrawal of the rejection is therefore, respectfully requested.

Claims 1, and 3-5 have been rejected as being anticipated by or obvious over Birkenmeyer et al. (U.S. Patent No. 5,667,974). Birkenmeyer et al. is asserted to teach the use of the affinity reagents in kits of the present invention. The Examiner asserts Birkenmeyer et al. differ from the present invention in failing to claim kits containing the reagents. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

Birkenmeyer et al. is drawn to a method of detecting nucleic acids. There is no disclosure in Birkenmeyer et al. of a general kit for detecting molecules other than nucleic acid. Nor is there a disclosure of the principle by which the presently claimed kit functions, i.e. that simultaneous recognition of two or more determinants are required to amplify the signal. As such, the present

invention is clearly not anticipated by or obvious over Birkenmeyer et al. Withdrawal of the rejection is therefore, respectfully requested.

Rejections under 35 U.S.C. §103

Claims 1 and 3-5 have been rejected under 35 U.S.C. §103 as being obvious over Nickerson et al, Delahunty et al., Kwok et al. or Nilsson et al. The cited references are asserted to teach the reagents of the present invention. The Examiner asserts it would be obvious to include the reagents in the presently claimed kits. Applicants traverse this rejection and withdrawal thereof is respectfully requested.

Firstly, Nickerson et al. regards the sequencing of DNA to identify genetic polymorphisms. Clearly, DNA sequencing has no relationship to the present invention for detecting macromolecules. The disclosure of Nickerson et al. references the oligonucleotide ligation assay (OLA) of Delahunty et al. and Kwok et al. Both the OLA assay and the "padlock" probes of Nilsson et al. regard methods of analyzing DNA sequences. In the references, ligation is used to distinguish differences in the DNA sequences that the probes have hybridized to. In the present invention, it is the binding of the affinity probes which juxtaposes

the oligonucleotides, which can then be amplified. With the present invention, oligonucleotide ligation is a means of detecting closely located probe-binding sites. This concept is completely different than that of Nickerson et al, Delahunty et al., Kwok et al. or Nilsson et al., where DNA sequences are analyzed through sequence ligation. There is no sequence analysis with the present invention. There is no suggestion in Nickerson et al, Delahunty et al., Kwok et al. or Nilsson et al. of detecting macromolecules, such as protein antigens, through the simultaneous binding of two or more probes as detected by the ligation of oligonucleotides. As such, the present invention is clearly not obvious over Nickerson et al, Delahunty et al., Kwok et al. or Nilsson et al.

Claims 1-2 and 6 have been rejected under 35 U.S.C. §103 as being obvious over Lee et al. in view of Dattagupta et al. (U.S. Patent No. 4,748,111). Lee et al. is asserted to disclose an immunometric assay using an immobilized antibody and two or more soluble antibodies. Lee et al. is asserted to differ from the present invention in failing to teach the use of olidgonucleotides as the affinity reagent.

Dattagupta et al. is asserted to teach the use of nucleic acids as carriers for labels in immunoassays. The

Examiner asserts it would have been obvious to use nucleic acid labels of Dattagupta et al. in the assay of Lee et al.

Applicants traverse this rejection and withdrawal there is respectfully requested. Lee et al. disclose a sandwich immunoassay, wherein two antibodies bind to a single antigen, forming a "sandwich." The "sandwich" assay format is a commonly employed principle in immunoassays. However, sandwich assays suffer from the limitation that nonspecific bound antibodies (Ab_3 of the Figures) contribute to the signal, reducing the assay sensitivity. The present invention is drawn to a kit and assay method which require the simultaneous binding of two or more affinity probes, for a signal to be created. This requirement of simultaneous binding of multiple affinity probes eliminates the problems associated with background signal created by non-specific binding.

The disclosure of Dattagupta et al. merely teaches the conjugation of oligonucleotides to a protein. There is no suggestion in Dattagupta et al. of improving assay sensitivity by requiring the simultaneous binding of at least two probes to create a signal. By using the teachings of Dattagupta et al. one skilled in the art would achieve the same sandwich assay of Lee et al. except having oligonucleotide-conjugated antibodies as probes. As such,

the assay achieved by combining the teachings of Lee et al. and Dattagupta et al. would have the same sensitivity problems caused by the same non-specific binding as present in the assay of Lee et al. alone. Lee et al. and Dattagupta et al., therefore, do not combine to result in the present invention. Nor is the present invention in any way suggested by the combination of these references. Withdrawal of the rejection is, therefore, respectfully requested.

Claims 3-5 and 7-8 have been rejected under 35 U.S.C. §103 as being obvious over Lee et al. in view of Dattagupta et al. (U.S. Patent No. 4,748,111) and in further view of Ciechanover et al. (U.S. Patent No. 5,026,653). Ciechanover et al. is further asserted to teach the use of antibodies detectably labeled with DNA for detecting antigens through immuno-PCR reactions or ligase chain reactions. The Examiner asserts it would have been obvious to use the teachings of Ciechanover et al. to modify the assay of Lee et al./Dattagupta et al. and use the amplification of the DNA to readily detect the antibody binding.

Applicants traverse this rejection and withdrawal there is respectfully requested. The method Ciechanover et al. is the same as that disclosed in Sano et al. (Science 258: 120-122(1992)), i.e. a method of detecting specifically bound antibodies by amplifying a nucleic acid sequence conjugated to an antibody. However, as with Lee et al. combined with Dattagupta et al., as discussed above, Ciechanover et al. also fail to disclose the simultaneous binding of two or more probes to generate a signal and thus improve the sensitivity of the assay. As such, the present invention is not achieved or suggested by combining Lee et al. with Dattagupta et al. and Ciechanover et al. Withdrawal of the rejection is, therefore, respectfully requested.

As the above-presented amendments and remarks address and overcome the rejections of the Examiner, withdrawal of the rejections and reconsideration and allowance of the claims are respectfully requested. Should the Examiner have any questions regarding the present application, he is requested to contact MaryAnne Liotta, PhD (Reg. No. 40,069) in the Washington DC area, at (703) 205-8000.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or

credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. §§1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

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